

AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Please amend the specification by replacing the paragraph beginning at page 7, line 6, with the following paragraph:

Figures 3a and 3b show the sequences of two self-cleaving RNAs (SEQ ID NO:21 in Figure 3a and SEQ ID NO:23 in Figure 3b) and the corresponding DNAs (SEQ ID NO:20 in Figure 3a and SEQ ID NO:22 in Figure 3b) that can be used as mutagenesis enhancers.

Please amend the specification by replacing the paragraph beginning at page 7, line 8, with the following paragraph:

Figure 4 shows a representative example of a mutagenic mini-exon sequence (SEQ ID NO:24) that can be used in conjunction with the presently described vectors.

Please amend the specification by replacing the paragraph beginning at page 7, line 11, with the following paragraph:

Figure 5 shows a variety of synthetic exon sequences (SEQ ID NOS:25-33) that can be used in conjunction with the described 3' gene trap cassette.

Please amend the specification by replacing the paragraph beginning at page 13, line 25, with the following paragraph:

In a particularly preferred embodiment, the described vectors employ a 5' gene trap cassette that comprises a selectable marker gene preceded by a splice acceptor sequence and followed by a polyadenylation (pA) sequence (SA β geoPA, Figure 2). Alternatively, SAires β geoPA can be used which further incorporates an internal ribosome entry site upstream from the β geo gene, or SAneoPA can be used (which dispenses with the β -gal activity). The above 5' gene trap cassettes can efficiently mutate genes and can be used to follow the expression of the trapped gene. Optimizing the SA sequence used can further enhance, or regulate the efficiency of the 5' gene trap cassette. Examples of suitable SA sequences include, but are not limited to:

GCAACCAGTAACCTCTGCCCTTCTCCATGACAACCAGGT (SEQ ID NO:[] 1); GATGATGTCATACTTATCCTGTCCTTTTTCCACAGCT (SEQ ID NO:[] 2); GGCGGTCAGGCTGCCCTCTGTTCCCATTGCAGGAA (SEQ ID NO:[] 3); TGTCAGTCTGTCATCCTGCCCTTCAGCCGCCGGATGGCG (SEQ ID NO:[] 4); TGCTGACACCCCCTGTTCCCTGCAGGACCGCCTTCAAC (SEQ ID NO:[] 5); TAATTGTGTAATTATTGTTTTCTCCTTAGAT (SEQ ID NO:[] 6); CAGAATCTTCTTTAATTCTGATTTATTCCTATAGGA (SEQ ID NO:[] 7); TACTAACATTGCCTTCCCTCCCTCCCACAGGT (SEQ ID NO:[] 8); TGCTCCACTTGAAACAGCTGTCTTCTTGCAGAT (SEQ ID NO:[] 9); CTCTCTGCCTATTGGTCTATTTCCCACCCCTAGGC (SEQ ID NO:[] 10); and ATTAATTACTCTGCCATTCCCTCTTCAGAGTT (SEQ ID

NO:[] 11). Any of the above SA sequences can be used in conjunction with, for example, SAnoopA or SAIREneopA.

Please amend the specification by replacing the paragraph beginning at page 62, line 17, with the following paragraph:

RNA isolation was carried out on DNA bind plates (Corning/Costar) treated with 5'-amino (dT)₄₂ (GenoSys Biotechnologies) in a 50 mM Sodium Phosphate buffer, pH 8.6, and allowed to sit at room temperature overnight. Immediately prior to use the plates were rinsed three times with PBS and twice with TE. Cells were rinsed with PBS, lysed with a solution containing 100 mM Tris-HCl, 500 mM LiCl, 10 mM EDTA, 1% LiDS, and 5 mM DTT in DEPC water, and transferred to the DNA binding plate where the mRNA was captured. After a 15 minute incubation the RNA was washed twice with a solution containing 10 mM Tris-HCl, 150 mM LiCl, 1 mM EDTA, and 0.1% LiDS in DEPC water. The RNA was then rinsed three times with the same solution minus LiDS. Elution buffer containing 2 mM EDTA in DEPC water was added and the plate was heated at 70°C. for five minutes. An RT premix containing 2x First Strand buffer, 100 mM Tris-HCl, pH 8.3, 150 mM KCl, 6 mM MgCl₂, 2 mM dNTPs, RNAGuard (1.5 units/reaction, Pharmacia), 20 mM DTT, QT primer (3 pmol/rxn, GenoSys Biotechnologies, sequence

5'CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTT3', SEQ ID NO:[] 12) and Superscript II enzyme (200 units/rxn, Life Technologies) was added. The plate was transferred to a thermal cycler for the RT reaction (37°C[.] for 5 min., 42°C[.] for 30 min., and 55°C[.] for 10 min.).

Please amend the specification by replacing the paragraph beginning at page 63, line 12, with the following paragraph:

The cDNA was amplified using two rounds of PCR. The PCR premix contains:

1.1× MGBII buffer (74 mM Tris pH 8.8, 18.3 mM Ammonium Sulfate, 7.4 mM MgCl₂, 5.5 mM 2ME, 0.011% Gelatin), 11.1% DMSO (Sigma), 1.67 mM dNTPs, Taq (5 units/rxn), water and primers. The sequences of the first round primers are: P₀, 5' AAGCCCGGTGCCTGACTAGCTAG3', SEQ ID NO: [[_____]] 13; BTK₀, 5' GAATATGTCTCCAGGTCCAGAG3', SEQ ID NO: [[_____]] 14; and Q₀, 5' CCAGTGAGCAGAGTGACGAGGAC3', SEQ ID NO: [[_____]] 15 (pmol/rxn). The sequences of the second round primers are P₁ 5' CTAGCTAGGGAGCTCGTC3', SEQ ID NO: [[_____]] 16; BTK₁ 5' CCAGAGTCTTCAGAGATCAAGTC3', SEQ ID NO: [[_____]] 17; and Q₁ 5' GAGGACTCGAGCTCAAGC3', SEQ ID NO: [[_____]] 18 (50 pmol/rxn).

The outer premix was added to an aliquot of cDNA and run for 17 cycles (95°C[[.]] for 1 min., 94°C[[.]] for 30 sec., 58°C. for 30 sec 65°C[[.]] for 3.5 min). An aliquot of this product was added to the inner premix and cycled at the same temperatures 40 times.

Please amend the specification by replacing the paragraph beginning at page 64, line 8, with the following paragraph:

Dye terminator cycle sequencing reaction with AmpliTaq® FS DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, Calif.) were carried out using 7 pmoles of primer (Oligonucleotide OBS; 5' CTGTAAAACGACGGCCAGTC3', SEQ ID NO: [[_____]] 19) and approximately 30-120 ng of 3' RACE product. The cycling profile was 35 cycles of 95°C[[.]] for 10 seconds, 55°C[[.]] for 30 seconds, and 60°C[[.]] for 2

minutes. Unincorporated dye terminators were removed from the completed sequencing reactions using G-50 columns as described earlier. The reactions were dried under vacuum, ~~resuspending~~ resuspended in loading buffer, and electrophoresed through a 6% Long Ranger acrylamide gel (FMC BioProducts, Rockland, [[Me.]] ME) on an ABI Prism.RTM. 377 with XL upgrade as per the manufacturer's instructions.